Original Research Article

Effect of vitamin E on serum antioxidant enzymes in traumatic brain injury-induced rats.

ABSTRACT

Objective;Traumatic brain injury (TBI) represents one of the major causes of mortality and disability in the world. This study was designed to investigate the role of antioxidants in the treatment of induced TBI in albino rats.

Methodology; Adult albino rats were induced with TBI by weight drop method. Rats were grouped into three groups of eight rats each. Group I served as traumatized-treated group (TT), group II served as non-traumatized, non-treated group (TNT) and group III served as normal control group. The treatment group received 67.5mg/kg of vitamin E (VE). Treatment started 30 minutes after trauma and continued for 21 days. Antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx)] and malondialdehyde (MDA) in serum tissue were assayed to evaluate oxidative stress (OS).

Results; The treated group showed a significant ($p < 0.05$) increase in the activities of antioxidant enzymes (SOD, CAT GPx) and significant ($p<0.05$) decrease in concentration of MDA compared to the TNT group.

Conclusion; Conclusively, these promising results suggest that the use of antioxidant VE may be useful neuroprotective strategy in the treatment of TBI.

Key words; Oxidative stress, Traumatic brain injury, Antioxidant, Malondialdehyde.

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1. INTRODUCTION

Traumatic brain injury (TBI) is characterized by alterations in brain function or immediate physical damage to brain tissue caused by an external force, followed by subsequent biochemical processes that can worsen the injury [1]. The severity of the damage isn't always immediately apparent and depends on various factors, including whether the external force was accidental or intentional, a direct impact or acceleration/deceleration, or the result of blast forces, and whether it involved penetration or not. Every year, around 70 million individuals suffer from TBI, with over 8 million people suffering from TBI-related disability[2]. In the United States alone, around 2.3 million cases of TBI occur yearly [3], with over 64,000 of these cases resulting in mortality, equating to nearly 176 TBI-related deaths every day [4]. Unfortunately, TBI is more common in developing nations [5]. The degree of brain injury can vary from minor to severe, and even seemingly mild injuries can lead to life-threatening complications. Projections for the prevalence of TBI in Africa are substantial, with an estimated 6 to 14 million new cases expected by 2050 [6]. Road traffic accidents (RTA) are the major cause of head and spinal cord injuries in Africa, with head injuries being the most common among all injuries in Nigeria [7].RTA is responsible for 80% of all injuries in Nigeria alone [8], with an annual incidence rate of 2710/100,000 [7]. The high occurrence in Nigeria is thought to be related to a lack of adherence to safety norms as well as a poor road network [9]. Concussions, penetrating injuries, closed head injuries, skull fractures, hematomas, lacerations, anoxia, contusions, and diffuse axonal injuries (DAI) may result from the impact exerted by external forces on the brain [10]. This impairs normal cellular function of the brain and may be present in all injury severities (mild, moderate, and severe) [11].

Based on the mechanism of injury, TBI is classified into two: primary injury, which occurs immediately after impact, and secondary injury, which is derived from cellular and biochemical changes that occur hours or days after trauma [12].TBI severity is determined by primary injuries [13], which alter the structural integrity of the brain tissue, resulting in vascular and parenchymal damage, intracerebral haemorrhage, and axonal shearing [14].

Secondary injuries aggravate the damage caused by TBI [13], through a cascade of mechanisms that leads to long-term or life-long difficulties [14] and even mortality [15]. Mechanisms such as hypoxia, glutamate excitotoxicity, disruption of the blood brain barrier (BBB), calcium overload, mitochondrial dysfunction, and neuro-inflammation all contribute to cell death via necrosis or apoptosis [15, 16,]. Of all these mechanisms, TBI is believed to be chiefly caused by the interplay between glutamate excitotoxicity, Ca2^{+} excess, and oxidative stress [12]with oxidative stress being the major cause of neuronal cell death.

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One of the well-studied and established aspects of secondary injury to brain tissue is the generation of free radicals following TBI [17]. A free radical is any chemical specie capable of independent existence with one or more unpaired electron which is responsible for its reactivity [18]. Reactive oxygen species (ROS) and reactive nitrogen specie (RNS) comprise both free radicals and compounds that can decompose to generate free radicals. ROS and RNS are frequently produced after TBI through numerous mechanisms. These reactive species are involved in the pathogenesis of TBI by worsening other secondary injury mechanisms and stimulating oxidative stress [19].

Oxidative stress has been incriminated as a potential contributor to the pathogenesis of acute central nervous system injury. After brain injury, the spontaneous generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) leads to tissue damage via various cellular and molecular pathways. Radicals can cause damage to lipids, proteins, and nucleic acids (e.g. DNA), resulting in oxidative stress and subsequent cell death [17]. Mammalian cells possess intracellular or endogenous antioxidants such as superoxide dismutase, catalase or glutathione peroxidase, in order to protect the cells against excessive levels of free radicals [20].

Antioxidants are chemicals that prevent the oxidation of other chemicals. They protect the key cell components by counteracting the damaging effect of free radicals [21].

The first and most important line of antioxidant defense systems against ROS is the enzymatic antioxidant (SOD, CAT, GPx) [22]. While these endogenous antioxidants increase their level of activity afterTBI, the magnitude of increase in free radicals compromised the antioxidant system to neutralize the adverse effects of free radicals [17].

However exogenous addition of compounds with antioxidant property such as vitamins, minerals (selenium, zinc), or other compounds like albumin can provide additional protection [23]. These natural antioxidants or other compounds that can neutralize free radicals may be of central importance in the prevention of oxidative stress.

Vitamin E, a potent peroxyl radical scavenger, is a chain-breaking antioxidant that prevents the propagation of free radical damage in biological membranes [24, 25]. Vitamin E has a potent function in improving immune system, stress, and disease resistance [25]. The aim of this research work is to validate the neurochemical role of vitamin E in TBI.

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2. MATERIALS AND METHODS

2.1 Animals

All the experimental rats were apparently healthy albino rats weighing 200-250g.They were obtained from the Animal House of the Biological Sciences Department, University of Maiduguri, Nigeria for this study. The rats were allowed to acclimatize to the research laboratory condition and were subjected to a 12 hours light/12 hour dark schedule. The rats were fed with growers' mash of vital® feed and allowed to clean drinking water *ad libitum*.

2.2 Experimental Design

The experimental animals were randomly divided into 3 groups. Group I (induced & treated with VE) group II-traumatized but not treated (TNT), and group III normal control group (that is non-traumatized non-treated (NTNT). The treatment lasted for 21 days. This work was approved by the board of University of Maiduguri after meeting national and international standard. Care of animals used was in accordance with institution guidelines.

2.4 Induction of TBI

Head injury was induced in the entire experimental animals except in the negative control group by weight drop method using an acceleration impact device of Marmarou [26].

2.5 Sample collection

The rats were anesthetized using chloroform in a glass jar and blood was collected by cardiac puncture and serum was harvested for biochemical analysis

2.6 Analysis of oxidative Stress

Oxidative stress markers were assayed in the serum tissue. The antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and lipid peroxidation byproduct malondialdehyde (MDA) were assayed using Cayman's Assay Kits, with batch number 706002 for SOD, 707002 for CAT, 703102 for GPX and 700870 for MDA. The manufacturer's instructions were carefully followed.

2.7 Statistical Analysis

Results were analyzed using the statistical package - SPSS version 22. Results were expressed as means ± SD. Data were analyzed by one-way analysis of variance (ANOVA). If the F values were significant, the Tukey post-hoc test was used to compare groups.

3. RESULTS

3.1. The Effect of Supplementation of TBI Rats with Vitamin E on the Activity of Serum SOD

Figure. 1 shows the result of serum SOD level in antioxidant treated groups. The result indicated that TBI caused significant ($P<0.05$) decrease in the activity of the enzyme. Supplementation of the antioxidant at 67.5 mg/kg body weight (BW) increased the SOD activity significantly ($P<0.05$).

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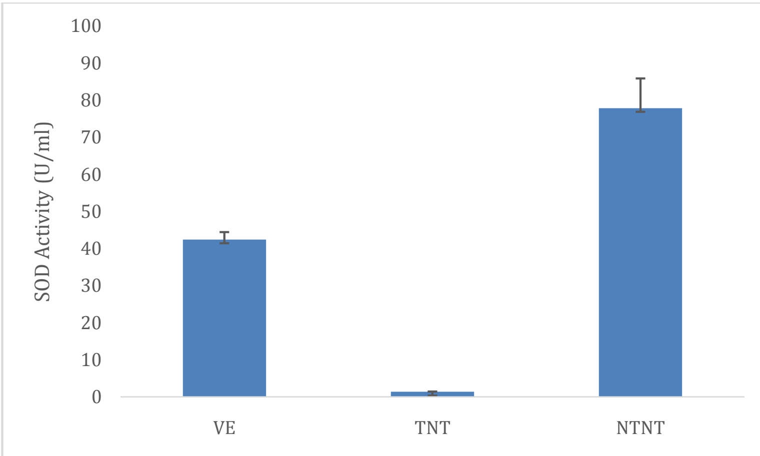

Figure 1: Effects of VE on the Activity of Superoxide dismutase in Blood;
*-SOD- Superoxide dismutase, TNT- Traumatized non- treated, NTN- Non-traumatized non- treated, VE – Vitamin E *(p<0.05).*

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3.2. The Effect of Supplementation with Vitamin E on the Activity of Serum CAT

Figure 2 shows the outcome of supplementation with VE on the activity of CAT in TBI rats. The result indicated that TBI caused significant (P<0.05) decrease in the activity of the enzyme. Supplementation of the antioxidant at 67.5 mg/kg body weight increased the activity significantly (P<0.05).

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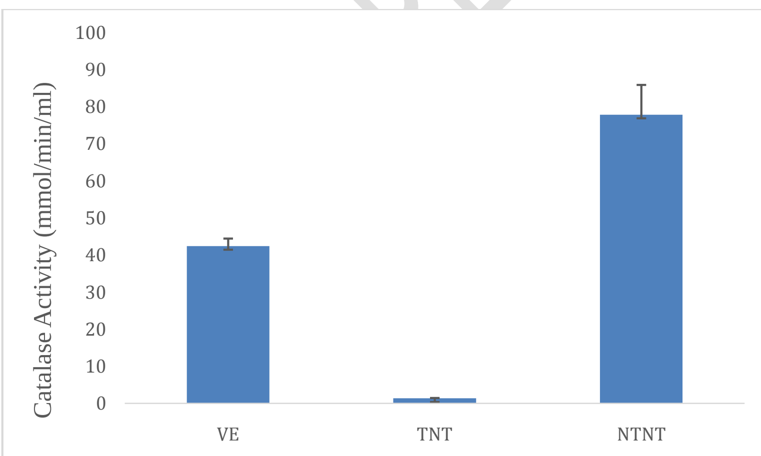

Figure 2: Effects of VE on the Activity of CAT in Blood
;-CAT- Catalase, TNT- Traumatized non- treated, NTN- Non-traumatized non-treated, VE – vitamin E Values with asterisk are significantly different (p<0.05) (p<0.05)*

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3.2. The Effect of Supplementation with VE on the Activity of Serum GPx

The effects of VE on the activity of GPx was presented in Fig. 3. The results showed that TBI caused significant (P<0.05) decrease in the activity of the enzyme. Administration of the antioxidant at 67.5 mg/kg BW, significantly (P<0.05) increased the activity.

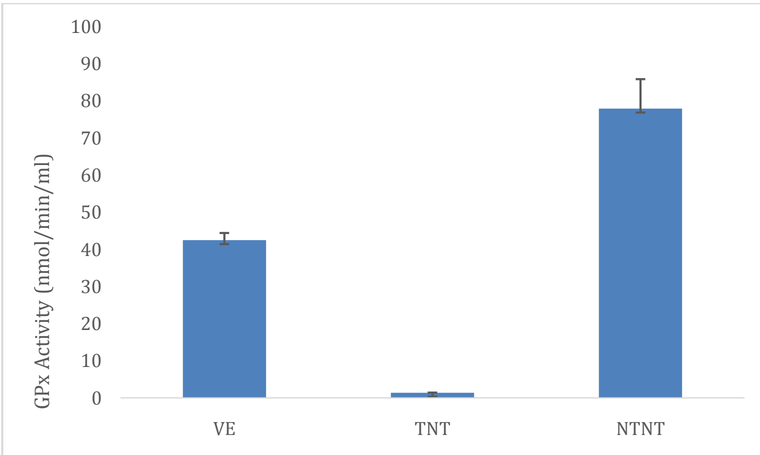

Figure 3: Effects of VE on the Activity of GPX in Blood

;-GPX- Glutathion peroxidase, TNT- Traumatized non- treated, NTN- Non-traumatized non- treated, VE – vitamin E (p<0.05)

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3.3. *The Effect of Supplementation with VE on the Serum Concentration of MDA*

Figure 4 shows the effects of LMWA on lipid peroxidation. The results indicated that TBI causedsignificant (P<0.05) increase in the concentration of MDA in the TNT group. After supplementation with VE at 67.5mg/kg, the concentration of MDA decreased significantly (P>0.05).

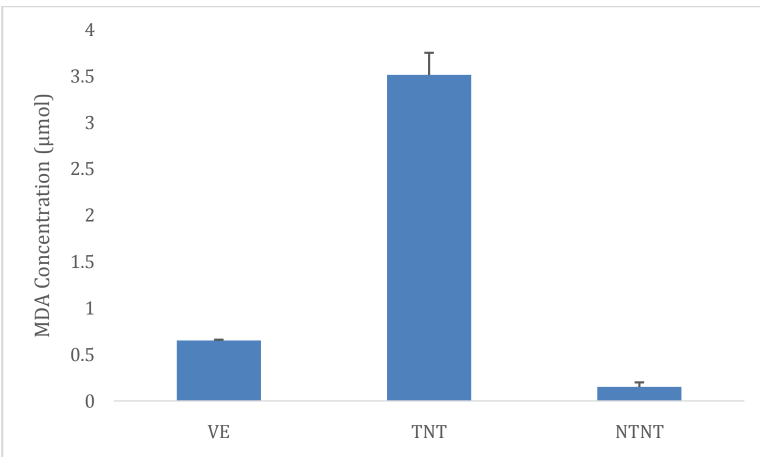

Figure 4: Effects of VE on the Concentration of MDA in the Blood of experimental rats*; MDA- Malondialdehyd, TNT- Traumatized non treated, NTN- Non-traumatized non- treated, VE – vitamin E. (p<0.05*)

4. DISCUSSION

Oxidative stress in this study was evaluated by measuring the levels of SOD, CAT, GPx and MDA as indicators of enzymatic antioxidant activity and lipid peroxidation respectively. A significant decrease was observed in the activities of SOD, CAT, GPx and increase in concentration of MDA in the serum tissue of TNT rats as compared to NTNT rats (Figure 1-4).This suggests the occurrence of OS due to the induced TBI. Supplementation with two different dose of DMSO, ALA, V C, V E, Mannitol and UA ameliorated the induced OS in dose depended manner (Figure 1-4).

Treatment of TBI-induced rats with 67.5 mg/kg of VE indicated significant increase (P< 0.05) in the activities of SOD, CAT and GPx and decrease concentration of MDA in the serum tissue of the treated group compared to the TNT group (Figure 1 – 4). This might be due to the ability of VE to quench free radicals and reduce their oxidative activity on lipids, proteins and nucleic acid which leads to the suppression of the antioxidant system and accumulation of MDA.

It can also be due to the regeneration of vitamin E and glutathione which are very effective against ROS [27]. This might be attributed to the characteristics of vitamin E as the most relevant chain-breaking antioxidant and abundance in cells and mitochondria membrane where ROS are also generated and their dysfunction causes excessive release of free radicals. Therefore it may have acted by inhibiting lipid

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peroxidation and OS in these important sites of free radical generation as reported by Inci and colleagues [28]. Jean-MARC [29], reported that vitamin E is a lipid-soluble antioxidant which prevents the formation of lipid peroxide. Also the modifying effect of vitamin E on OS pathways and improving neurological outcome have been reported in many animal studies [28]. It is also known that apart from its direct effect on ROS, vitamin E can react with various antioxidants such as vitamin C, GSH, β-carotene to bring about synergistic activity. In return these antioxidants regenerate vitamin E. there by potentiating its effect [30].

The findings of this work revealed that the group treated with VE have significantly ($P<0.05$) increased activities of the antioxidants enzymes and significantly ($P<0.05$) decreased level of MDA compared to the TNT counterpart (Figure 1– 4).

5. CONCLUSION

In conclusion, after TBI induction, significant decrease was observed in antioxidant enzymes activities with concomitant increase in MDA level indicating the occurrence of oxidative stress. Oxidative stress is the strategic pathogenic mechanism of secondary injury that results in neuronal degeneration and functional deficits, mitigating this damaging process is neuroprotective and restores the neuronal function. The antioxidant given have indicated its neuroprotective and neurorestorative potential by boosting the antioxidant capacity and inhibiting lipid peroxidation. These promising results suggest that vitamin E may be useful in the management of TBI.

ETHICAL STATEMENT

The work described in this study was carried out in accordance with *The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving animals.*

This work was approved by the board of University of Maiduguri after meeting national and international standard. Care of animals used was in accordance with institution guidelines

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